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Cellular antioxidant and antiproliferative activity of ethanolic extract of *Coleus tuberosus*

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Ethanolic extract of both the flesh and peel of *Coleus tuberosus* (EEPC) were evaluated for cellular antioxidant activity based on 2,7-diacetyl dichlorofluorescein (DCFH) oxidation, and antiproliferative activity based on 3-(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide (MTT) assay, using human breast cancer MCF-7 cells. The result showed that antioxidant and antiproliferative activity of EEPC was higher than EEFC. Its bioactive compounds ursolic acid was higher than oleanolic acid. The antioxidant and antiproliferative activities were in a dose-dependent manner. The cellular antioxidant and antiproliferative activities of EEFC and EEPC were observed to be related with both oleanolic and ursolic acid contents of the flesh and peel of C. tuberosus. These results indicate that EEFC and EEPC and their bioactive compounds reduced phorbol miristate acetate (PMA)-induce oxidative stress; and might be used as a potential source of natural antioxidants and antiproliferative agents.

Key words: Antioxidant, antiproliferative, *Coleus tuberosus*; ursolic acid, oleanolic acid.

INTRODUCTION

Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen, and hydrogen peroxide are often generated as by-product of biological reactions or from exogenous factors. *In vivo*, some of these ROS plays positive role such as energy production, phagocytosis, regulation of cell growth and intracellular signalling, or synthesis of biologically important compounds. However, ROS may also be very damaging. They can attack lipids in cell membranes and also attack DNA, including oxidations that cause membrane damage and also cause DNA mutation leading to cancer.

Oxidative stress is an imbalance between the production of ROS and antioxidant defense, and may lead to oxidative damage. It can result from a deficiency in antioxidant defense mechanism, or from an increase in ROS, due to exposure to elevated ROS levels, the presence of toxins metabolized to ROS or excessive activation of ROS systems, such as, those mediated by chronic infection and inflammation. In addition to endogenously produced antioxidants and enzymes that catalyze the metabolism of ROS, ROS can be scavenged by exogenously obtained antioxidants from fruits and vegetables. Plants (fruits, vegetables, medicinal herbs, etc.) contain a wide variety of free radical scavenging molecules, such as phenolic compounds (for example,
phenolic acids, flavonoids, anthocyanins and tannins), nitrogen compounds (alkaloids, amines and betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity (Zheng and Wang, 2001; Cai et al., 2003). Epidemiological studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities to a greater or lesser extent (Mclarty, 1997; Owen et al., 2000). The intake of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and diseases associated with ageing. Antioxidant activity is a fundamental important property for human life. Many of the biological functions, including antimutagenicity, anticarcinogenicity, and antiaging, among others, originate from this property (Yang et al., 2008). These compounds also have antioxidant and anti-free-radical properties that allow them to quench free radicals in the body (Chitindingu et al., 2007). Moreover, it was reported that antioxidants with ROS scavenging ability have great relevance in the prevention of oxidative stress which is responsible for the majority of diseases, including cancer.

Coleus tuberosus is tuber vegetables of the family Lamiaceae. One of the characteristics of bioactive compounds in plants belonging to the family Lamiaceae is the presence of triterpenic acid. Triterpenic acid in ethanolic extract of C. tuberosus was oleanolic acid and ursolic acid (Nugraheni et al., 2010). There is growing interest in the elucidation of the biological and pharmacologic roles of the plant-derived triterpenic acid compound, in term of antioxidant, hepatoprotective, analgesic, antitumor, anti inflammatory, and immunomodulatory effect (Yon and Liu, 2008; Feng et al., 2008; Fai and Tao, 2009). However, the potential health benefit of C. tuberosus related with cellular antioxidant and antiproliferative activity has not been studied to date. The objectives of this study were to evaluate cellular antioxidant and antiproliferative activity of ethanolic extract of C. tuberosus on MCF-7 cancer cells.

MATERIALS AND METHODS

Materials

Ursolic acid was obtained from Santa Cruz Biotech Inc., Dulbecco’s Modified Eagle Medium (DMEM), oleanolic acid, 2,7-diacetate dichlorofluorescein, (DCFH-DA) and Phorbol Mirstate Acetate (PMA) were obtained from Sigma-Aldrich, fetal bovine serum (FBS) was obtained from Gibco. MCF-7 cells were obtained from American Type Culture Collection (ATCC). All other reagents and solvents were of analytical reagent grade.

Preparation of Coleus tuberosus extracts

C. tuberosus was obtained from farmer in Bantul District, Yogyakarta Special Region, Indonesia at commercial maturity, 3 months. Peel (±1 mm) of C. tuberosus was obtained by peeling using potato peeler. Peel and flesh of C. tuberosus were cut into small pieces and dried with cabinet dryer at 40°C for 24 h, blended into powders. Preparation of crude extract (Mooi et al., 1999) of 17 samples was macerated with ethanol 1:5 at room temperature for a week. The crude extract were then filtered through Whatman No.1 filter paper, dried by water bath and evaporated in vacuum rotary evaporator at 45°C. The extract was stored at -20°C for further analysis. The ethanol peel and flesh from C. tuberosus were named ethanol extract of peel of C. tuberosus (EEPC) and ethanol extract of flesh of C. tuberosus (EEFC).

Determination of triterpenic acid (ursolic and oleanolic acids) content by high performance liquid chromatography (HPLC) analysis

HPLC analysis was performed on HPLC apparatus equipped with Eurosphere 100-5 photodiode array detector (Shimadzu Corporation, Kyoto Japan). Separation were carried out at 30°C on Eurosphere 100-5 C-18 column (5 micron, 250 × 4, 6 mm). The HPLC assay for quantitative determination of ursolic and oleanolic acids in EEPC and EEFC was carried out as described by Du and Chen (2009). Elution was performed at room temperature (23°C), humidity 55% and utilized the mobile phase consisted of a mixture, methanol (MeOH): 0.15% CH3COOH (90:10), monitored by wavelength 210 nm, and the flow rate was 1 ml/min. Extract was dissolved in 1 ml methanol, filtered with millex 0.45 µM and injected into HPLC. The samples and the standards were injected at a volume of 20 µl each. Ursolic and oleanolic acids were identified by comparison of their retention time (tR) values and ultraviolet (UV)-visible spectra with those of known standards and were quantified by peak areas from the chromatogram. Determination of ursolic and oleanolic acids contents were based on standard curve.

Cell culture

Human breast cancer MCF-7 cells were obtained from ATCC. Cells were cultured in the DMEM, supplemented with 10% heat-inactivated FBS and penicillin (100 units/ml-streptomycin (100 µg/ml), using 75 cm² flasks in a 37°C in humidified 5% CO₂ incubator.

Reduction of oxidative stress in MCF-7 cells

In this study, the effect of EEFC, EEPC, ursolic and oleanolic acids on reduction of oxidative stress in human breast cancer MCF-7 cells was evaluated on the basis of the method reported by Chang et al. (2001) and Wolfe and Liu (2007). DCFH-DA, a peroxide-sensitive dye, was used for the evaluation of oxidative stress in cells based on oxidation of DCFH-DA by ROS (Figure 1) and modulation from Wolfe et al. (2007). In this study, MCF-7 cells (human breast cancer cell line) were obtained from the ATCC (Rockville, MD). The cells were cultured in DMEM supplemented with 10% FBS, 100 units/ml penicillin, and streptomycin in an incubator at 37°C, 5% CO₂, 95% air, and humidity. The cell suspensions (200 µl at the concentration of 10⁵ cells/well) were seeded in and incubated with ursoic acid (5, 10, 20 and 40 µg/ml), oleanolic acid (10, 20, 40, and 80 µg/ml), EEFC and EEPC (100, 200, 400 and 800 µg/ml) for 20 min. Then cells were co-incubated with 25 µM DCFH-DA in the absence or presence of 100 ng PMA in darkness at 37°C for 30 min. After incubation, cells were collected and washed once with ice-cold phosphate buffered saline (PBS), re-suspended in 200 ul of the same PBS, and placed on ice in darkness until flow cytometry was carried out. The amounts of intracellular hydrogen peroxide were detected by BD flow
Figure 1. Method and proposed principle of the cellular antioxidant activity assay. Cells were pretreated with antioxidant compounds or vegetables extracts. The antioxidant bound to the cell membrane and or passed through the membrane to enter the cell. Cell treated with DCFH and PMA. DCFH-DA diffused into the cell where cellular esterases cleaved the diacetate moiety to form the more polar DCFH, which was trapped within the cell. Cell were treated with PMA, which was able diffuse into cells. PMA spontaneously decomposed to form peroxy radical. The peroxy radicals attacked the cell membrane to produce more radicals and oxidized the intracellular DCFH to the fluorescent DCF. Antioxidant prevented oxidation of DCFH and membrane lipids and reduce the formation of DCF.

Antiproliferation assay

Cell culture

Human breast cancer MCF-7 cells were obtained from ATCC. Cells were cultured in the DMEM, supplemented with 10% heat-inactivated FBS and penicillin [100 units/ml-streptomycin (100 µg/ml)], using 25 cm² flasks in a 37°C in humidified 5% CO₂ incubator.

Cell viability assay

3-(4,5-Dimethylthiazol-2)-2,5-diphenyltetrazolium bromide (MTT) assay was performed according to the method of Hogan et al. (2010), with little modification. MCF-7 cells were plated into 96-well microtiter plates at a density 1.5 × 10⁴/well in a final volume of 100 µl culture medium per well. The cells were treated with EEFC and EEPC (700 to 1000 µg/ml), Ursolic acid (5 to 20 µg/ml), oleanolic acid (40 to 70 µg/ml) and maintained at 37°C with CO₂ for 48 h. After the incubation period, 10 µl of MTT labelling reagent (5 mg/ml) was added to each well. The microtiter plate was then incubated again for 4 h at 37°C with 5% CO₂. Then, 100 µl of the solubilisation solution was added into each well. The plate was allowed to stand overnight in the incubator at 37°C and 5% CO₂. The cell viability
Table 1. Triterpenic acid content in EEFC and EEPC.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ursolic acid (µg/g sample)</th>
<th>Oleanolic acid (µg/g sample)</th>
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<tbody>
<tr>
<td>EEPC</td>
<td>13.78 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.75 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>EEFC</td>
<td>3.41 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.71 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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</table>

Values are expressed as mean ± standard deviation (n = 3). Means with different letters in the same column were significantly different at level of p < 0.05.

Figure 2. EEFC inhibited ROS generation in dose dependent manner in MCF-7 cells was evaluated using the DCFH-DA assay. Data are presented from three independent experiments. Different letters are significantly different (P < 0.05) compared to control (no treatment).

Effective reduction of oxidative stress in MCF-7 cells

DCFH-DA, a free-radical sensitive indicator, was used for evaluating the effectiveness of EEFC, EEPC, ursolic and oleanolic acids on the oxidative stress in mammalian cells based on the method reported by Chang et al. (2001) upon oxidation of the non-fluorescent DCFH-DA molecule by ROS; For example, H₂O₂, the 2,7-dichlorofluorescein (DCF) molecules are generated as products that emit green fluorescence. The fluorescence intensity relevant to the H₂O₂ concentration can thus be measured quantitatively by using flow cytometric analysis. Figures 2, 3, 4 and 5 show that an decrease of oxidative stress in human breast cancer MCF-7 cells was detected when performed by HPLC. Using a standard curve of ursolic and oleanolic acids, the amount of ursolic and oleanolic acids in EEPC was calculated to be 13.78 ± 0.15 and 19.75 ± 0.30, for ursolic and oleanolic acids, respectively. EEFC, 3.41 ± 0.04 and 3.71 ± 0.06 µg/g sample, for ursolic and oleanolic acids, respectively (Table 1).
the cells were treated with 100 ng/ml PMA, EEFC, EEPC, ursolic and oleanolic acids relative to the untreated cells (PMA alone).

Levels of 79.74 ± 0.72, 81.79 ± 0.45, 84.54 ± 1.01 and 84.96 ± 0.22% reduction of oxidative stress in 100 ng/ml PMA treated MCF-7 cells were observed when co-incubated with 100, 200, 400 and 800 µg/ml of EEFC, respectively (Figure 2). Levels of 85.26 ± 0.95, 91.72 ± 0.20, 92.07 ± 0.64 and 92.24 ± 0.63% reduction of oxidative stress in 100 ng/ml PMA treated MCF-7 cells were observed when co-incubated with 100, 200, 400 and 800 µg/ml of EEPC, respectively (Figure 3).
Levels of 79.36 ± 2.12, 86.37 ± 1.25, 88.30 ± 1.10 and 90.85 ± 1.28% reduction of oxidative stress in 100 ng/ml PMA treated MCF-7 cells were observed when co-incubated with 5, 10, 20 and 40 µg/ml of ursolic acid, respectively (Figure 4). Levels of 63.82 ± 3.00, 65.80 ± 5.29, 75.22 ± 1.85 and 80.26 ± 2.04% reduction of oxidative stress in 100 ng/ml PMA treated MCF-7 cells were observed when co-incubated with 10, 20, 40 and 40 µg/ml of oleanolic acid, respectively (Figure 5).

The reduction of oxidative stress of MCF-7 treated with EEPC is higher than EEFC, and its bioactive compounds: ursolic acid is higher than oleanolic acid. These results demonstrate that the levels of oxidative stress in MCF-7 cells were effectively reduced when the cells were treated with EEFC, EEPC, ursolic and oleanolic acids, suggesting that the damage from oxidative stress in test cells could be greatly alleviated by EEFC, EEPC, ursolic and oleanolic acids.

Antiproliferative activity of EEFC, EEPC, ursolic and oleanolic acids

Figures 6, 7, 8 and 9 show the effects of EEFC and EEFC on proliferation human breast cancer MCF-7 cells. The antiproliferative activity of EEPC and EEFC on MCF-7 cells were characterized by conducting MTT assay. Cells were treated with 700, 800, 900 and 1000 µg/ml of extracts and incubated for 48 h. These results indicated EEFC at highest concentration: 1000 µg/ml at 48 h, viability of MCF-7 cells was 30.316% (Figure 7). Treatment with ursolic acid 20 µg/ml, viability of MCF-7 cells were 12.756% (Figure 8) and treatment with oleanolic acid 70 µg/ml, viability of MCF-7 cells were 42.458% (Figure 9).

It was also shown that at all treatments, had the percentage of growth inhibition activity dose-dependent manner. The antiproliferative activity of EEPC and EEFC were expressed as the half maximal inhibitory of concentration (IC50). Since the lower the IC50 value indicated the higher antiproliferative effect of the sample, IC50 growth inhibition after incubation 48 h of EEFC is 965.31 ± 3.39. EEPC: 812.22 ± 5.72 ml, ursolic acid = 3.81 ± 0.18, oleanolic acid 48.61 ± 0.90 (Table 2). This study showed that EEPC had the antiproliferative effect higher than EEFC at all concentrations which corresponds with its lower IC50.

DISCUSSION

Principle of cellular antioxidant activity in this research, (Figure 1) the probe, DCFH-DA, is taken up by MCF-7 human breast cancer cells and deacylated to DCFH. Peroxyl radicals generated from PMA lead to the oxidation of DCFH to fluorescent DCF, and the level of fluorescence measured upon excitation is proportional to the level of oxidation. Vegetables or fruits extracts and pure phytochemical compounds quench peroxyl radical or ROS and inhibit the generation of DCF. Thus, cellular
Treatment with ethanolic extract of flesh of *C. tuberosus* 48 h (µg/ml)

**Figure 6.** Effect of EEFC on the proliferation of MCF-7 cells for 48 h. EEFC inhibited the proliferation of MCF-7 cells in dose-dependent manners. Different letters are significantly different (P < 0.05) (B). Data are presented as mean ± SD from three independent experiments.

Treatment with ethanolic extract of peel of *C. tuberosus* 48 h (µg/ml)

**Figure 7.** Effect of EEPC on the proliferation of MCF-7 cells for 48 h. EEPC inhibited the proliferation of MCF-7 cells in dose-dependent manners, different letters are significantly different (P < 0.05) (B). Data are presented as mean ± SD from three independent experiments.
Figure 8. Effect of ursolic acid on the proliferation of MCF-7 cells for 48 h. Ursolic acid inhibited the proliferation of MCF-7 cells in dose-dependent manners. Different letters are significantly different (P < 0.05) (B). Data are presented as mean ± SD from three independent experiments.

Figure 9. Effect of oleanolic acid on the proliferation of MCF-7 cells for 48 h. Oleanolic acid inhibited the proliferation of MCF-7 cells in dose-dependent manners. Different letters are significantly different (P < 0.05) (B). Data are presented as mean ± SD from three independent experiments.

Antioxidant activity assay uses the ability of peroxyl radicals or ROS to induce the formation of a fluorescent oxidative stress indicator in the cell culture and measures the prevention of oxidation by antioxidants (Wolfe and Liu, 2007).

The antioxidant capacity of EEFC, EEPC, ursolic and oleanolic acids was evaluated against ROS under biological medium by using a cellular test. The DCFH-DA
was used to evaluate the intracellular redox status. The ROS are high energy forms of oxygen; they induce hyperoxidation, cytotoxicity of oxygen and they decrease the antioxidant activity (Tarnawski et al., 2006; Khochevar and Redmond, 2000). Polyphenolic, flavonoid, anthocyanin and tannin compounds have high antioxidant properties, and their effects are significant on human nutrition and health.

It has been reported that the body’s antioxidant defense system consists of the activity of superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glutathione (GSH) (Bandyopadhyay et al., 1999). SOD catalyzes the breakdown of endogenous cytotoxic superoxide radicals to \( \text{H}_2\text{O}_2 \) which is further degraded by CAT. Thus, they play a crucial role in maintaining the physiological levels of \( \text{O}_2 \) and \( \text{H}_2\text{O}_2 \). GSH, in conjunction with GST, has a basic role in cellular defense against deleterious free radicals and other oxidant species (Arivazhagan et al., 2000). GST catalyzes the conjugation of thiol group of GSH to electrophilic substrates and thereby detoxifies endogenous compounds such as peroxidized lipids.

In this study, MCF-7 cells was induced by PMA in order to increase the amount of ROS mainly \( \text{H}_2\text{O}_2 \) in MCF-7 cells. Induced by PMA in MCF-7 cells led to the increase amount of ROS that have an impact on the increasing occurrence of oxidative stress. Sztawrowski and Nathan (1991) reported that induction of PMA in cancer cells result in an increased number of ROS in cancer cells mainly type \( \text{H}_2\text{O}_2 \).

The effect of EEFC, EEPC, and its bioactive compounds such as ursolic and oleanolic acids on oxidative stress in MCF-7 cells, induced by the addition PMA, was investigated using flow cytometry analysis with DCFH-DA, a free radical sensitive indicator. When DCFH-DA is oxidized by ROS, for example, \( \text{H}_2\text{O}_2 \), it is converted to DCF and emits green fluorescence. The fluorescence intensity, related to the \( \text{H}_2\text{O}_2 \) concentration, can thus be measured quantitatively by flow cytometric analysis (Muanda et al., 2011).

In the present study, we show that EEFC and EEPC possessed the ability to scavenge free radicals and reduce oxidative stress in human breast cancer MCF-7 cell lines (Figures 2 and 3). Ability of EEPC greater than EEFC to decreased PMA-induced oxidative stress in MCF-7, its related with the greater ursolic and oleanolic acids contents in EEFC than EEPC (Table 1) and another phytochemical such as phytosterol, phenol and flavonoids. Phytochemical studies of EEFC and EEPC revealed that its bioactive compounds such us ursolic acid, oleanolic acid and another phytochemical such as phytosterol, phenol and flavonoids contribute to the antioxidant activities of this vegetables (Mooi et al., 2010). These compounds are reported for the first time from this plant. The evidence from this study suggests that EEFC, EEPC and its bioactive compounds such as ursolic and oleanolic acids could be used as a food supplement for human health care. These results also suggest that the damage from oxidative stress in test cells could be greatly alleviated by EEFC, EEPC, ursolic and oleanolic acids.

### Table 2. IC\(_{50}\) treatment of ursolic acid, oleanolic acid, EEFC and EEPC on MCF-7 cells after incubation for 24, 48 and 72 h.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC(_{50}) (µg/ml)</th>
<th>48 h</th>
</tr>
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<tbody>
<tr>
<td>Ursolic acid</td>
<td>3.81 ± 0.184(^a)</td>
<td></td>
</tr>
<tr>
<td>Oleanolic acid</td>
<td>48.61 ± 0.9(^b)</td>
<td></td>
</tr>
<tr>
<td>EEFC</td>
<td>965.31 ± 3.39(^d)</td>
<td></td>
</tr>
<tr>
<td>EEPC</td>
<td>812.22 ± 5.72(^c)</td>
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Values are expressed as mean ± standard deviation (n = 3). Means with different letters in the same column were significantly different at level of p < 0.05.
the mRNA expression of CAT might tend to increase the protein synthesis, which in turn leads to increase in the activity of the enzyme. Since CAT preferably quenches \( \text{H}_2\text{O}_2 \), ursolic acid might elicit its antioxidant potential at the level of CAT. Further, since CAT is synthesized by microbodies and hepatocytes contain large number of it, administration of ursolic acid might have increased the proliferation rate of microbodies, thus increasing their synthesis. Thus, though all three enzymes contribute to the antioxidant defense system, it can be stated that the mechanism of action of ursolic acid is mainly mediated or targeted towards increasing the levels of CAT.

The biologically active antioxidant phytoconstituents found in *Ficud hispida* (such as oleanolic acid) restoration enzyme antioxidant defense system by increased tissue levels of SOD, CAT, GPX and GSH and non-enzyme antioxidant defense system such as Vitamin C, Vitamin E and beta carotene in Azathioprine (AZA) administrated rat (Jung et al., 2008; Kim et al., 2005; Li et al., 2007).

The free radical scavenging and antioxidant property of ursolic acid have been recently proved by Dufour et al. (2007). It was thought that this antioxidant property is due to the polyphenolic methyl group present in ursolic acid (Zhang et al., 2001). Pre-treatment with ursolic acid in UVB-irradiated lymphocytes increased the activities of SOD, CAT and thus ursolic acid could exert a beneficial action against pathological alteration caused by the UVB-radiation. Further, the increase in the activity of SOD, CAT, GPX and GSH in UVB-irradiated lymphocytes is mainly because of the antioxidant sparing action of ursolic acid (Mortin-Aragon et al., 2001). Pre-treatment with ursolic acid prior to irradiation protected vitamin C and vitamin E depletion resulting from the radiation effect. These results show that ursolic acid renders protection against UVB-radiation induced oxidative stress (Ramachandran et al., 2008).

Lin et al. (2007) reported that after ethanol extract of *Ligustrum lucium* fruits (ELL) treatment which have oleanolic acid and ursolic acid as the major bioactive compounds, a significant increase in the levels of antioxidant enzymes such as SOD, CAT and GPX with a corresponding decrease in the level of lipid peroxides in the liver, kidney and lung. This finding supports the protective effect of ELL against butylated hydroxytoluene (BHT)-induced oxidative stress.

Thus, the discussed facts, suggest that ursolic and oleanolic acids participate in restoring the reduction environment of a cell which might also be a contributing factor in restoration of cellular functions.

EEFC, EEPC, ursolic and oleanolic acids caused significant growth inhibition of MCF-7 cells in dose-dependent manner (Figures 6 to 9). The sensitivity of MCF-7 breast cancer cells to EEFC, EEPC, ursolic and oleanolic acids is characterized by \( \text{IC}_{50} \) value (Table 2). These results indicate that elevated antiproliferative effects are strengthened with dose of exposure.

This study showed that EEPC had the antiproliferative effect higher than EEFC at all concentrations which corresponds with its lower \( \text{IC}_{50} \). The inhibition of cancer cell proliferation by EEFC and EEPC can be partially explained by the triterpenic acid content mainly ursolic and oleanolic acids. Ursolic and oleanolic acids were responsible for their antiproliferative activities. The higher antiproliferative effect of EEFC compare to EEPC was related with the higher of ursolic and oleanolic acids content in the peel than the flesh (Table 1).

In this study, we examined whether ursolic acid as bioactive compounds in *C. tuberosus* had strong inhibitory effect on the proliferation of MCF-7 cells in dose-dependent manner compare with oleanolic acid, which corresponds its lower \( \text{IC}_{50} \). Previous studies showed that ursolic acid had strong inhibitory effect on the proliferation and induce apoptosis in a number of cancer cells lines (Subbaramaiah et al., 2000; Shishodia et al., 2003; Shan et al., 2009; Zhang et al., 2007). But oleanolic acid, which is the isomer of ursolic acid have lower antiproliferative effect.

Shyu et al. (2010) indicated that oleanolic and ursolic acids could inhibit the growth of HuH7 human hepatocellular carcinoma cells with \( \text{IC}_{50} \) 100 and 75 \( \mu \text{M} \), respectively. Ursolic and oleanolic acids had anti-proliferative effect on Jurkat cell line (T cell lymphoma) and were suggest that ursolic and oleanolic acids have significant anti-tumor activity. Ursolic acid is most effective than oleanolic acid with \( \text{IC}_{50} \) value: 75 and 150 \( \mu \text{M} \). Li et al. (2002) reported that ursolic acid gives a higher percentage of antiproliferative effect than oleanolic acid. Presence of different substituent at different position of molecule alters the antiproliferative effect. Since both the compounds are region-isomers, the difference in their potency may be attributed to their structural arrangement of the substituent. It is methyl group at positions 19 and 20, which makes a difference in potencies of these compounds (Senthil et al., 2007). Based on the results obtained, *C. tuberosus* was found to serve as a potential source of food diet and bioactive compounds that can be as a cellular antioxidant and antiproliferation MCF-7 cancer cells. These results are consistent with the research of Imran et al. (2010) which reported that the *Morus* species (mulberry fruits) were found to serve as a potential source of food diet and natural antioxidants.

The EEPC, EEFC and its bioactive compounds (ursolic and oleanolic acids) are potential as cellular antioxidant which will be able to decreased PMA induced oxidative stress and inhibit proliferation in human breast cancer MCF-7 cells at a dose-dependent manner. Ursolic and oleanolic acids that are present in *C. tuberosus* may be partially responsible for the cellular antioxidant and antiproliferative activities of whole *C. tuberosus*.

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